

**Research Paper**

*Afr. J. Traditional,
Complementary and
Alternative Medicines*
www.africanethnomedicines.net

ISSN 0189-6016©2008**ANTI-NOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF ETHANOL EXTRACT OF *SYZYGIUM AROMATICUM* FLOWER BUD IN WISTAR RATS AND MICE****Tanko, Y¹, Mohammed, A.¹, Okasha, M. A.¹, Umar, A. H¹ and Magaji, R. A.²**¹Department of Human Physiology, ABU, Zaria.²Department of Human Physiology, Bayero University Kano.***Email:** yusuftanko @ yahoo.com**Abstract**

The ethanol extracts of *Syzygium aromaticum* flower bud were tested for anti-nociceptive and anti-inflammatory effects in mice and Wistar rats which were carried out using acetic acid-induced abdominal contractions in mice and formalin-induced hind paw edema in Wistar rats. Three doses of the ethanol extract (50, 100, and 200mg/kg body weight i.p.) were used for both studies. The extract had an LD₅₀ of 565.7 mg/kg body weight intraperitoneally in mice. The extracts produced significant effect ($P < 0.05$) at all the three doses. Similarly, the anti-nociceptive activity produced significant effects ($P < 0.05$) at all the three doses of the extract. The result supports the local use of the plant in painful and inflammatory conditions.

Key words: *Syzygium aromaticum* flower bud, anti-inflammatory, anti-nociceptive; Anti-Inflammatory; Acetic acid-induced abdominal constriction.

Introduction

Cloves is dried flower bud of *Syzygium aromaticum* Merr. and Perry. (Myrtaceae), an evergreen tree 10-20 m. in height indigenous to India, Indonesia, Zanzibar, Mauritius and Ceylon. They are reddish-brown in colour and have a strong aroma. Cloves has antiseptic, antibacterial, antifungal and antiviral properties (Blumenthal, 1998). The oldest recorded medicinal use of cloves was in China, where it was reported for various ailments as early as 240 BC. Cloves was taken over the centuries for diarrhea, liver, stomach and bowel ailments, and as a stimulant for the nerves (Gordon, 1980).

This research was aimed at investigating the possible anti-nociceptive and anti-inflammatory activities of ethanol extract of the plant, in order to support or refute the claims by traditional herbalists.

Materials and Methods**Collection and preparation of plant materials**

A sample of *Syzygium aromaticum* flower bud was bought commercially from Kaduna central market, Kaduna State, in June 2006. It was identified and authenticated by M. Musa of the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. Its voucher specimen number is 13954. The seeds were collected, dried and later ground into paste using laboratory mortar and pestle. The fine powder was extracted with ethanol for 72 h using a Soxhlet extractor. The percentage yield of the extract was calculated to be 18.2 % (w/w). The extract was reconstituted in distilled water at appropriate concentrations for the various experiments conducted.

Phytochemical screening

The ethanol extract obtained was subjected to preliminary phytochemical screening, to identify the chemical constituents. The methods of analysis employed were those described by Brain and Turner (1974).

Experimental animals

Adult Wistar rats of both sexes weighing between 160-190g and adult Swiss albino mice of both sexes weighing between 20-25g were used for the experiments. The animals were maintained under normal laboratory condition of humidity, temperature ($25 \pm 2^{\circ}\text{C}$) and light (12 h: 12 h night) for 7 days, and allowed free access to food and water *ad libitum*.

Acute toxicity study (LD₅₀)

This was conducted by using the method described by Lorke (1983). In the initial phase, mice were divided into 3 groups of three and treated with the ethanol extract of the plant at doses of 10.0, 100.0 and 1000.0mg extract/kg body weight intraperitoneally (i.p.) and were then observed for 24 hrs for signs of toxicity including death. In the final phase, mice were divided into 4 groups of one mouse each and treated with the ethanol extract at doses of 200, 400, 800 and 1600 mg / kg body weight i.p. The LD₅₀ was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose, i.e the geometric mean of the consecutive doses which 0 and 100% survival rates were recorded.

Anti-inflammatory activity

Adult Wistar rats were divided into 5 groups of 5 rats in each group. The first group served as negative control (normal saline i.p.) while the second, third and the fourth groups received different doses of the extract (50, 100 and 200 mg/kg body weight, i.p.) respectively while the fifth group received the reference drug Diclofenac (25mg/kg i.p.). Thirty mins later all the groups were administered 50 µl of a 2.5 % solution of formalin, subcutaneously under the plantar surface of the left hind-paw. An increase in the rats' hind paw linear diameter induced by subplantar injection of formalin was used as the measure of acute inflammation (Winter et al., 1963). The paw diameter was measured with the aid of a vernier caliper at 1, 2, 3, 4, and 5h, after the injection of formalin. The difference between the readings at time 1h and different time interval was taken as the thickness of edema (Hess and Milong, 1972).

$$\text{Inhibition (\%)} = \frac{\text{Mean paw diameter (control)} - \text{Mean paw diameter (treated)}}{\text{Mean paw diameter (control)}} \times 100$$

Analgesic activity

The test was carried out using the method of Koster et al. (1959). Different concentrations of the extract (50, 100, and 200mg/kg.) were given intraperitoneally. Thirty minutes after treatment, the mice were injected intraperitoneally with 0.6 % acetic acid solution to induce characteristic writhing. Piroxicam (20mg/kg i.p) was used as a reference drug while the control group received normal saline. Five minutes after Acetic acid injection, mice were placed in individual cages and the number of abdominal contractions was counted for each mouse for a period of 10 mins. Percentage inhibition of writhing was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{\text{Mean number of writhings (control)} - \text{Mean No. of writhings (test)}}{\text{Mean number of writhing (control)}} \times 100$$

Statistical analysis

All data were expressed as the mean \pm S.E.M. Data was subjected to ANOVA. Differences in means were considered to be significant when $P < 0.05$ as described by Duncan et al. (1977).

Results

Phytochemical screening of the extract revealed the presence of: flavonoids, resins, glycosides, tannins,

saponin and alkaloids. The signs of toxicity were first noticed after 2-6 hours of extract administration. There was decreased locomotor activity and sensitivity to touch and jerking. Also there was decreased feed intake, tachypnoea and prostration after 14 hrs of extracts administration. The median lethal dose (LD₅₀) in mice was calculated to be 565.7 mg/kg

The ethanol extract of *S. aromaticum* suppressed the paw oedema induced by formalin in rats as shown in Table 1. The extract produced 42, 45 and 52% inhibition respectively. The highest activity was obtained the dose of 200mg/kg. The mean latency of nociceptive responses to abdominal constriction is summarized in Table 2. The extract significantly exerted a protective effect on acetic acid-induced abdominal constriction. The extract produced 75, 66 and 65% inhibition respectively. The highest activity resides at the lowest dose of 50mg/kg.

Table: 1: Effect of ethanol extract *Syzygium aromaticum* flower bud on formalin-induced paw oedema in rats

Treatment groups (n=5)	Dose (mg/kg)	Oedema diameter(cm)				
		1h	2h	3h	4h	5h
Normal Saline	10ml/kg	0.734± 0.16	0.802±0.125	0.784±0.125	0.784± 0.1252	0.722± 0.144
<i>Syzygium aromaticum</i> Extract	50	0.422± 0.074 ^a	0.452±0.054 ^a	0.430±0.0402 ^a	0.424±0.038 ^a	0.416± 0.042 ^a
<i>Syzygium aromaticum</i> Extract	100	0.444± 0.042 ^a	0.416±0.072 ^a	0.408±0.0715 ^a	0.402± 0.693 ^a	0.398± 0.067 ^a
<i>Syzygium aromaticum</i> Extract	200	0.424± 0.074 ^a	0.358±0.063 ^a	0.352± 0.058 ^a	0.350± 0.603 ^a	0.350± 0.603 ^a
Diclofenac	25	0.59± 0.045 ^{ns}	0.61± 0.045 ^a	0.61± 0.0224 ^a	0.63± 0.447 ^a	0.64± 0.045 ^a

Each value is mean ±SEM of 5 rats ^ap<0.05; compared to control NS: statistically not significant
Figures in parentheses represent percentage inhibition of anti-inflammatory activity.

Table 2: Effect of ethanol extract of *Syzygium aromaticum* Flower bud given intraperitoneally on acetic acid-induced abdominal Writhing contraction in mice

Treatment (i.p.)	Dose (mg/kg)	No. of abdominal Contractions	% inhibition
Norma saline	10ml/kg	23.8 ±2.41	-
<i>Syzygium aromaticum</i>	50	6.0 ±0.77 ^c	75%
<i>Syzygium aromaticum</i>	100	8.0±0.70 ^a	66%
<i>Syzygium aromaticum</i>	200	8.25±3.01 ^b	65%
Piroxicam	20	6.3 ±1.80 ^c	74%

Values of abdominal contraction are mean ± SEM. n =5

Values are statistically significant compared to control

Discussion

The effect of the ethanol extracts of *S. aromaticum* on anti-inflammatory and analgesic activities

were evaluated. The results obtained showed that the extract at the doses of 50 100 and 200mg/kg significantly reduced the number of acetic acid-induced writhes in mice. The activities reside more at the lowest dose of 50mg/kg body weight. The acetic acid induced writhing method, also called abdominal contraction response is a very sensitive one as it can detect ant-nociceptive effects of substances at a dose that may be inactive in other methods such as tail flick test (Collier et al., 1968). Abdominal constriction responses were found to partly involve local peritoneal receptors as it was postulated by Bentley et al.(1981), thereby suggesting that the extract of *S. aromaticum* flower bud could interfere with such peritoneal receptors to bring about the observed analgesic effect. The ethanol extract of *S. aromaticum* flower bud also demonstrated a significant ($P<0.05$) anti-inflammatory activity against formalin-induced oedema in rats at doses of 50mg/kg, 100mg/kg and 200mg/kg. The presence of flavonoids and tannins might be responsible for the anti-nociceptive and anti-inflammatory activities as flavonoids and tannins of were found to inhibit phosphodiesterases (Duke, 2002), which are involved in cell activation, whose effect depend upon on the biosynthesis of protein cytokines that mediate adhesion of circulating leucocytes to the site of injuries. Flavonoids and tannins have been proven to potently inhibit prostaglandins, a group of powerful inflammatory substances (Manthey, 2000).

The association of both anti-nociceptive and anti-inflammatory effects is well documented for various non steroidal anti-inflammatory agents (NSAIDS) (Gyires et al., 1985). The extract may be suppressing oedema formation by reducing vascular permeability as in case of NSAID (Swingle et al., 1974). Thus the plant could be used to serve as a potential source of anti-inflammatory and anti-nociceptive agents, which also supports the local claim of its use in painful and inflammatory conditions.

Acknowledgements

The authors of this work wish to acknowledge the technical assistance of Bala Mohammed and Nura Mohammed of the Department of Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria.

References

1. Bentley, G.A., Newton S.H., and Starr, J. (1981) Evidence from an action of morphine and the enkephallins can sensory nerve endings in the mouse peritoneum, *Brit.J.Pharmacol*, **79**:125-134.
2. Blumenthal, M. (1998). The Complete German Commission E Monographs: *Therapeutic Guide to Herbal Medicines*. Austin: American Botanical Council
3. Brain, K. R., and Turner, T.D. (1975) The Practical Evaluation of Phytopharmaceuticals, Wright *Scientifica*, Bristol, pp.57-58.
4. Collier, H.O., J. Dinneen, L.G., Johnson C.A. and Schneider (1968). The abdominal constriction response and its suppression by analgesic drugs in the mouse, *Brit.J. Pharmacol*; **32**:295-310.
5. Duke, J. A. (2002). CRC Handbook of medicinal herbs. (CRC Med Herbs ed2)
6. Friedman, M., Henika, P.R. and Mandrell, R.E. (2002). Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *Journal of Food Prot*; **65**(10):1545-60.
7. Gordon L. A. (1980). Country Herbal. Devon, England: Webb & Bower (Publishers) Ltd.
8. Gyires, K., Furts, S., Miklya, A., Budavari I., and Knoll, J. (1985). Drugs under Experimental and Clinical Research **2** (8): 493-500.
9. Swingle, K.F., Scherrer, R.R. (1974). Anti-inflammatory agents: *Chem.Pharmacol.* **2** :1129-1135.
10. Koster, R., Auderson, N., and De Beer, J.M. (1959). Acetic acid used for analgesic screening. *Federation Proceedings*; **18**:412.
11. Manthey, J.A. (2000). Biological properties of flavonoids pertaining to inflammation. *Journal of Microcirculation*; **7**(6): 29-34
12. Hess, S.M., and Milong R.C. (1972) *Inflammation Mechanism and Control*. Lepow. and Ward, P.A. ed, academic Press New York .p1-2.
13. Lorke, D. (1983). A new Approach to Practical Acute Toxicity Testing, Analgesic Screening, *Arch. Toxicol*, **53**:275-287.
14. Winter, E.A., and Nuss, G.V. (1963) Anti- Inflammatory and antipyretic activities of indomethacin. *J. Pharmacol. Exptl Ther.* **141**: 369-376.
15. Duncan, R. C; Knapp, R. G and Miller, M. C. (1977). Test of Hypotheses in population Means, In: *Introductory Biostatistics for Health Sciences*, John Wiley and Son, Inc. N. Y. pp. 71 – 76.